PATENT
MSB-7213

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	PETRA BOYLE GAYLE D. WETZEL KENNETH J. LEMBACH	DECLARATION UNDER 37 C.F.R. § 1.132
Serial No.:	08/145,060	EXAMINER: R. D. BUDENS
Filed:	October 29, 1993	ART UNIT: 1806
For:	HUMAN ANTI-TNF ANTIBODIES	

Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Matthias Wabl, declare as follows:

1. I have been awarded a Ph.D. degree in Biology from the Max Planck Institute, Berlin and have approximately 16 years experience in making cell lines that express monoclonal antibodies.

2. UTILITY: The above-entitled Patent Application is concerned with human monoclonal antibodies that specifically bind to TNFα. I understand the Examiner has rejected the claims in that Patent Application on the ground that Applicants have not mentioned specific uses for the antibodies. In my opinion, a variety of specific uses would immediately be obvious to a person skilled in the art. For example, it is well known that any monoclonal antibody, once generated, can be used in a variety of immunoassays which would be inherently useful for not only research but as diagnostic tools. As shown in the enclosed catalog copies, anti-TNF antibodies are commercially available, thus confirming their obvious utility.

In addition, I am aware of clinical studies currently in progress using murine monoclonal antibodies that bind to TNFα. See the

attached copy of a Poster Session No. 696, presented at the 3rd ICAAC meeting, October 17, 1993. See also the enclosed copy of an article that appeared in the July 15, 1994, Genetic Engineering News showing that Chiron/Miles is developing an anti-TNF monoclonal antibody for the targeting of TNFα.

3. ENABLEMENT: I understand the Examiner states it is not clear from the teachings of the Patent Application that one of ordinary skill in the art could make other human anti-TNFα monoclonal antibodies that bind specifically to TNFα without undue experimentation. I have reviewed the Applicants' patent Application and claims and believe that one skilled in the art, given the disclosure of the Patent Application could duplicate the Applicants' work and generate other cell lines that express human monoclonal antibodies that bind specifically to TNFα without undue experimentation using known screening techniques.

The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States code and that such willful false statements may jeopardizethe validity of the Application or any patents issuing thereon.

Jan 24, 95
Date

Matthias Wabl, Ph.D.

3rd ICAAC
Oct 17-20 '93

Session 64. *Poster*
IMMUNE MEDIATORS
Tuesday, 10:30 A.M.

R.P WENZEL. Univ. of [redacted] ollege of Medicine, Iowa City, IA.

Because [redacted] cacy trials for anti-endotoxin therapy evaluate mortality at 1 mo[redacted] because short-term survival does not translate to long-term survi[redacted] evaluated post-discharge survival of 100 septic patients entered in a double-blind, placebo-controlled efficacy trial of monoclonal antiendotoxin antibody (XOMEN-E5) between 12/86 and 12/90 at our institution. Beginning in 5/92, we contacted all known survivors. We found that 59 deaths occurred (29/50 (58%)-drug group (E5) vs 30/50 (60%)-placebo group). Thirty-three (55%) patients died within the first month of the septic episode, 6 (10%) died within 3 months, and 4 (6%) died within 6 months. Five patients died within 1 year, 6 within 2 years and 4 within 3 years. An additional patient died 5 years after the initial sepsis. We examined which factors predicted long-term survival (up to 5 years). The largest univariate hazard ratios (HR) were associated with severity of underlying disease as classified by McCabe (rapidly fatal: HR=27.4, p=0.0001 and ultimately fatal disease: HR=8.3, p=0.024). Thus, the mortality rate of patients with rapidly or ultimately fatal underlying diseases was 27.4 and 8.3 times greater than that of patients with non-fatal disease. Age and weight had low, but significant HRs of 1.03 (p=0.0004) and 1.02(p=0.03). The presence of infection-associated morbidities predicted long-term survival: disseminated intravascular coagulation (HR=2.2, p=0.008), shock (HR=2.4, p=0.002), central nervous system dysfunction (HR=2.5, p=0.0008). Having acute respiratory distress syndrome and receiving E5 did not affect survival (p=NS). Sex, positive cultures from blood or other sterile body sites, or admission to ICU was not significant by univariate analysis. Multivariate models will be developed. Our data show that 45% of deaths occur after clinical trials are terminated. Important outcomes may be missed if clinical trials only use a1 month follow-up.

693 Placebo (Pla) Controlled Study of Amdigen® (AMP) in HIV Disease: Improvement in CD4 Level and Delayed Type Hypersensitivity (DTH)

K. THOMPSON[1], D. STRAYER[2], P. SALVATO[3], N. KLIMAS[4], A. MOLAVI[4], A. HAMILL[5], W. CARTER[1]. [1]HEM Pharmaceuticals Corp., Phila, PA; [2]Hahnemann University Hospital, Phila., PA; [3]CFIDS Center, Houston, TX; [4]VA Medical Center, Miami, FLA; [5]Nelson-Tobedo Clinic, Dallas, TX

CD4 lymphocyte levels are an important surrogate marker for HIV disease progression. DTH response, a marker of in vivo immune function, has also been reported to predict HIV progression and survival independently of CD4 count (Blinx, et al, 1989, V Int. Congress AIDS, Montreal pQ, Th.B.P.155; Gordin, et al, 1992, Prog. Abstr. 32nd ICAAC, Anaheim CA, p. 335). The effect of AMP on these two endpoints was examined in a 48 week, double-blind, Pla-controlled study. 36 AZT-treated (≥ 5 months), HIV+ patients (pts) with CD4 levels of 100-500 were stratified by baseline CD4 and AZT usage and randomly assigned IV infusions of 400 mg AMP BIW (n=10); 700 mg AMP BIW (n=12); or Pla BIW (n=14). All Pts continued AZT therapy (300-500 mg/day). The groups were well-matched at baseline for sex, age, CD4 level, and duration of AZT therapy. 79% Pla and 73% AMP pts completed the study (p=1.0). Logistic regression analysis revealed that AMP-treated pts had a greater chance of positive DTH reactivity post-treatment than did Pla pts (p = .042). During the first 6 months, AMP pts reported fewer/less severe episodes of night sweats (p<.005). AMP treated pts that entered the study with CD4 counts > 300 (n=9) lost significantly fewer CD4 cells than Pla (n=6) over the course of the study (p=.04); AMP pts with CD4 < 300 did not differ from placebo. As part of a cross-over design, 7 of 11 eligible Pla pts began AMP therapy with resultant increased mean CD4 (p=.02) and DTH (p=.04) responses. These results indicate that AMP therapy modulates positively two markers of HIV disease progression and survival.

696 Monoclonal Antibody to Human Tumor Necrosis Factor (TNF MAb): Multi-center Efficacy and Safety Study in Patients with the Sepsis Syndrome. J. WHERRY, R. WENZEL,* R. WUNDERINK, H. SILVERMAN, T. PERL, S. NASRAWAY, H. LEVY, R. BONE, R. BALK, R. ALLRED, and the TNF MAb Study Group

TNF MAb (Bay x 1351) is a murine monoclonal antibody raised against human tumor necrosis factor. In experimental models it has been shown to be effective in protecting animals from the morbidity and mortality associated with sepsis induced by Gram Negative Bacteria or Staphylococcus aureus. To evaluate the efficacy and safety of TNF MAb in patients with sepsis syndrome, a large multi-center 3 arm clinical trial was conducted in 31 hospitals in North America. Patients with sepsis were prospectively stratified by shock/non-shock and randomized to receive a single intravenous dose of 15 mg/kg TNF MAb, 7.5 mg/kg TNF MAb, or placebo (0.25% human albumin). All patients received standard medical and surgical care and were closely followed with clinical and laboratory measures of efficacy and safety; survival or non-survival was determined over the 28 day study period.

After the first 800 patients were enrolled a planned interim analysis was performed using the intent-to-treat principle. Based only on preliminary survival data it was concluded that if the study were to continue as initially designed there would be insufficient power to support efficacy of TNF MAb at either dose, for all sepsis syndrome patients. However, among shock patients there was a trend towards efficacy with lower mortality rates in both active treatment arms with the greatest effect seen in the 15 mg/kg arm. Among non-shock patients TNF MAb did not appear beneficial.

The study is now complete and the full database for all 971 infused patients is being finalized. Comprehensive results will be presented

694 [redacted]

697 Double-blind, Randomized Comparison of the Safety Profile of Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) vs Recombinant Granulocyte Colony Stimulating Factor (G-CSF) in Advanced HIV Infected Patients (P) with Neutropenia. P. HERMANS*, P. FRANCHIOLY, N. CLUMECK. St Pierre University Hospital, Brussels, Belgium.

This pilot study was initiated to determine the toxicity profile of 2 CFSs for acute salvage therapy in advanced HIV P with an absolute neutrophil count (ANC) < 1000/mcl. Starting daily dose was 1mcg/kg by subcutaneous route. The target value for response was defined as an ANC > 1000/mcl. Results: 12 P were enrolled in each arm between 01/92 and 08/92. Demographic data, clinical background and ANC at baseline were similar for P with GM-CSF and G-CSF. Haematologic response was achieved in 9/12 and 12/12 respectively after a median of 3 days. Toxicity profile is summarized on the following table:

Adverse events (AE)	GM-CSF	G-CSF	P value
Fever	[redacted]	[redacted]	P<0.05
Flulike/myalgia	3	1	NS
Bone pain	0	1	NS
Skin reaction	1	0	NS
Eosinophilia > 20%	2	0	NS
At least one AE	10	[redacted]	P<0.001

We conclude that for an efficacy at least similar, G-CSF appears significantly better tolerated when compared with GM-CSF in neutropenic HIV patients.

fluctuations in glucose levels. How-

$400-800 Million Septic Shock Market by '97

A $400 million—and possibly $800 million—market for drugs to treat septicemia and septic shock is likely to emerge by the end of 1997, according to a new study by Frost & Sullivan, Inc., which is based Mountain View, CA.

Products under development include monoclonals, receptors, inhibitors, immunoconjugates and immunomodulators, miscellaneous proteins and peptides and low molecular-weight organic compounds.

The report ("U.S. Septicemia and Septic Shock Markets—The Search for Therapy Continues: New Agents and Their Potential") projects likely introduction dates for the range of septicemia and septic shock treatments in clinical trials as of the end of 1993. In each case, revenue projections for each drug are based on the assumption that a drug will be approved for use in the U.S. by the start of 1997.

Advanced in Development

Sales of two monoclonal products advanced in development and likely to receive approval in 1995—Chiron's T88 and Miles' Bay x-1351—are projected to reach $189 million by 1997. Besides T88, Ribi's MPL and Xoma's BP-23, also anti-endotoxins, could be approved by the end of 1996, with the three anti-endotoxins producing 1997 sales of nearly $140 million.

Chiron/Miles' Bay x-1351, a monoclonal that targets tumor necrosis factor, also could reach the market within this time frame and generate $135 million in revenue by 1997, according to the report. Anti-neutrophil revenues in 1997 are projected at $187 million, including sales of Repligen/Lilly's anti-CD11b, Cytel's CY-1787 and Scios Nova's NPC 15669 monoclonals, all expected to reach the market by the end of 1996.

The incidence of septicemia is increasing with growth of the more susceptible population segments—immunocompromised and aged persons—in the absence of any improvement in prevention or treatment. Despite the advent of potent new antibiotics, notes the report, the incidence of septicemia and septic shock has risen steadily for the past 30 years, and the high mortality rate associated with these conditions has persisted.

The key competitive issue will be drug efficacy, although safety is crucial as well since septic shock patients are extremely vulnerable by definition and anything that aggravates their condition in any way can easily cause death.

POLYCLONAL RABBIT ANTI-HUMAN TNF-α (NEUTRALIZING)

Ordering Information

Code: *IP-300*

Size: *1 mL*

Specification Summary

Antigen: *Purified recombinant human TNF-α (hTNF-α)*

Host Species: *Rabbit*

Antibody Class: *Primarily IgG and IgM*

Purity: *Supplied as neat hyperimmune antiserum*

Diluent: *None*

Stabilizer(s): *None*

Preservative(s): *None*

Sterility: *0.22 µm sterile filtered*

Volume/Vial: *1 mL*

Physical State: *Frozen liquid*

SPECIFICITY

Species Specificity

This antibody binds human TNF-α and rat TNF-α (3) but does not bind mouse TNF-α. Use Genzyme's anti-mouse TNF-α antibodies for analysis of mouse samples. The cross reactivity of this antibody with TNF-α from species other than mouse and rat has not been tested.

APPLICATIONS

Use this antibody for neutralizing hTNF-α bioactivity, for immunoprecipitation, and for immunocytochemistry. Use of anti-hTNF-α has been published (1-6).

DILUTION INSTRUCTIONS

Dilution

Dilute with PBS or medium which is identical to that employed in the relevant assay system including carrier protein (0.1–1% BSA or 0.1–10% appropriate serum). *Failure to add carrier protein to diluted product will result in loss of activity.*

STORAGE AND STABILITY

This antibody as shipped is stable for 6 months at -20°C. This antibody diluted as instructed is stable for at least 1 week when stored at -20°C. *Avoid multiple freeze/thaw cycles by storage in appropriate aliquots.*

INSTRUCTIONS FOR USE

Recommended Concentration(s) for Use

Recommended concentrations for use are approximate values. A dose response assay should be performed to determine the optimal concentration for use in each application.

1. Neutralization: For neutralization of human TNF-α bioactivity, use ~10 µl of antibody to neutralize approximately 1000 units of TNF-α bioactivity observed in the standard L929 cell cytotoxicity assay (5,6).

2. ELISA: This antibody can be titrated for effective use as the second antibody in an ELISA (2).

3. Immunocytochemistry: This antibody was used on cryostat tissue sections at a 1:500 dilution. Titrate the antibody to optimize staining in different samples.

REFERENCES

1. Beezhold *et al., J. Immunol.*, **143**:3217 (1989).
2. Sharief *et al., New Engl. J. Med.*, **325**:467 (1991).
3. Chin *et al., J. immunol.*, 145:3669 (1990).
4. Oki *et al., Lymph. Cyt. Res.*, 10:273 (1991).
5. Fast *et al., Infect. Immunit.* 57:221 (1989).
6. Ju *et al., J. Immunol.*, 144:23 (1990).

Genzyme
1994 cat

X E IC N

POLYCLONAL RABBIT ANTI-HUMAN TNF-α (WESTERN BLOT)

Ordering Information

Code: *IP-310*
Size: *1 mL*

TNF

Specification Summary

Antigen: *Purified denatured recombinant human TNF-α (hTNF-α)*
Host Species: *Rabbit*
Antibody Class: *Primarily IgG and IgM*
Purity: *Supplied as neat rabbit hyperimmune antiserum*
Diluent: *None*
Stabilizer(s): *None*
Preservative(s): *None*
Sterility: *0.22 µm sterile filtered*
Volume/Vial: *1 mL*
Physical State: *Frozen liquid*

SPECIFICITY

Species Specificity

This antibody shows minor cross-reactivity with mouse TNF-α upon blotting 1 µg or more of mouse TNF-α. Use Genzyme's anti-mouse TNF-α antibodies for analysis of mouse samples. The cross-reactivity of this antibody with TNF-α from species other than mouse has not been tested.

APPLICATIONS

The reactivity of this antibody with human TNF-α enables researchers to employ Western blot analysis for identifying cytokine components of cell culture supernatants or biological fluids. This offers the advantage of utilizing two parameters for identification of human TNF-α. First, SDS-PAGE provides an approximation of molecular weight. Second, the immunoreactivity confirms identification of human TNF-α. Direct side-by-side comparison of electrophoretic mobility and immunoreactivity exhibited by "unknown" samples to those displayed by known human TNF-α standards allows positive identification of human TNF-α in experimental samples. This antibody has also been shown to be useful in immunocytochemistry.

DILUTION INSTRUCTIONS

Dilution

Dilute with PBS or medium which is identical to that employed in the relevant assay system including carrier protein (0.1-1% BSA or 0.1-10% appropriate serum). *Failure to add carrier protein to diluted product will result in loss of activity.*

STORAGE AND STABILITY

This antibody as shipped is stable 6 months at -20°C. It is stable for at least 1 week when diluted as instructed and stored at -20°C. *Avoid multiple freeze/thaw cycles by storage in appropriate aliquots.*

INSTRUCTIONS FOR USE

Recommended Concentration(s) for Use

Recommended concentrations for use are approximate values. A dose response assay should be performed to determine the optimal concentration for use in each application. For Western blot analysis, use anti-TNF-α at a dilution of approximately 1:250 for the detection of 10 ng of SDS-denatured and β-mercaptoethanol reduced human TNF-α.

IB

Rabbit (polyclonal) Anti-Human IL-8/NAP-1, Purified

	AB-05-001	100 µg	$250

Purity: ≥98%, affinity purified
Physical State: Lyophilized, sterile-filtered
Application: Neutralizing, Western Blot, ELISA
Recognition: Native and recombinant human IL-8; recognizes all natural MW species of hIL-8.
Specificity: Neutralizing

Mouse (monoclonal) Anti-Human IL-10

Isotype: IgG, kappa	AB-61-005	0.5 mg	$195
Clone: B-S10	AB-61-010	1.0 mg	$315

Purity: >99%, ion-exchange chromatography
Concentration: 1.0 mg/ml in PBS
Application: Neutralizing, Western Blot, ELISA
Recognition: Native and recombinant human IL-10; does not cross-react with murine IL-10. No cross-reactivity has been observed with IL-1, IL-2, IL-6, IL-8 or TNF-α.
Specificity: 60 pg neutralizes 50% of the activity of 1 pg of IL-10.

BioSource International 1993-1994

Mouse (monoclonal) Anti-Human Tumor Necrosis Factor-α (TNF-α)

Isotype: IgG, kappa	AB-16-005	0.5 mg	$195
Clone: B-C7	AB-16-010	1.0 mg	$315

Purity: ≥99%, affinity purified
Concentration: 1.0 mg/ml
Application: Neutralizing, Western Blot, ELISA
Recognition: Native and recombinant human TNF-α; does not cross-react with human TNF-β or murine TNF-α.
Specificity: 0.2 µg neutralizes 1 unit of TNF-α activity.

Rabbit (polyclonal) Anti-Human Tumor Necrosis Factor-α (TNF-α)

Purity: Antisera	AB-06-001	1.0 ml	$315

Application: Neutralizing, Western Blot, ELISA
Recognition: Native and recombinant human TNF-α

Cytokines

recombinant human TNF-α

Catalog Number	210-TA	
Pack Size	10 µg	50 µg
Form	Lyophilized with human serum albumin as a carrier protein	
Source	*E. coli*-expressed	
Purity	>97%, as determined by N-terminus analysis and SDS-PAGE visualized by silver stain	
Activity	Measured in a cytotoxic assay using the TNF-susceptible murine L-929 cell line in the presence of the metabolic inhibitor actinomycin D (Matthews, N. and M.L. Neale, 1987. *Lymphokines and Interferons, a practical approach*, Clemens, M.J., Morris, A.G., and A.J.H. Gearing, eds., IRL Press, p. 221). The ED_{50} for this effect is typically 0.02 - 0.05 ng/mL.	

available carrier-free (210-TA/CF)

R & D Systems

1994 cat

... and associated Antibodies

anti-human TNF-α
polyclonal neutralizing antibody

Catalog Number	AB-210-NA
Pack Size	1 mg
Form	Lyophilized from a sterile solution in PBS
Type	Goat IgG
Antigen	*E. coli*-expressed recombinant human TNF-α
Specificity	Neutralizes the biological activity of rhTNF-α. It will not neutralize the biological activity of rmTNF-α or rhTNF-β. However, < 5% cross-reactivity with rmTNF-α is seen on direct ELISAs and western blots.
Neutralization	0.02 - 0.04 µg/mL of the antibody will neutralize 50% of the biological activity due to 0.25 µg/mL of rhTNF-α.
ELISA	0.15 ng/well of rhTNF-α can be detected using an antibody concentration of 0.5 µg/mL.
Western blot	An antibody concentration of 1.0 µg/mL will allow visualization of 0.2 ng/lane of rhTNF-α.

anti-human TNF-α
monoclonal neutralizing antibody

Catalog Number	MAB210
Pack Size	500 µg
Form	Lyophilized from a sterile solution in PBS
Type	Mouse IgG_1
Antigen	*E. coli*-expressed recombinant human TNF-a
Specificity	Neutralizes the biological activity of rhTNF-α and membrane-bound hTNF-α (Aversa, G. *et al.*, 1993, J. Exp. Med. 177:1575). It will not neutralize the biological activity of rhTNF-β or rmTNF-α. In direct ELISA and western blot analysis, this antibody exhibits no cross-reactivity with rhTNF-β, rmTNF-α, rhsTNF RI, or rhsTNF RII. When immobilized on a microtiter plate, this antibody will capture recombinant as well as natural human TNF-α.
Neutralization	0.02 - 0.04 µg/mL of the antibody will neutralize 50% of the biological activity due to 0.25 µg/mL of rhTNF-α.
ELISA	0.15 ng/well of rhTNF-α can be detected using an antibody concentration of 0.5 µg/mL.
Western blot	An antibody concentration of 1.0 µg/mL will allow visualization of 2.0 ng/lane of rhTNF-α under non-reducing conditions and 20 ng/lane under reducing conditions.

AFFINITY PURIFIED ANTIBODIES NATIVE AND CONJUGATED

DESCRIPTION	PACK	GRADE	CODE	PRICE
Biotin	2mg	AFF	AU078	$100
	1mg	AFF-F	AF078	$140
	1mg	AFF-P	AP078	$140
	0.5mg	AFF-A	AA078	$160
Human α 1 Fetoprotein	0.5mg	AFF	AU035	$120
	0.5mg	AFF-F	AF035	$140
	0.5mg	AFF-P	AP035	$160
	0.5mg	AFF-A	AA035	$180
Human Chorionic Gonadotrophin (β HCG)	2mg	AFF	AU074	$180
	1mg	AFF-F	AF074	$140
	1mg	AFF-P	AP074	$160
	0.5mg	AFF-A	AA074	$180
Human C-Reactive Protein	2mg	AFF	AU044	$180
	1mg	AFF-F	AF044	$140
	1mg	AFF-P	AP044	$160
	0.5mg	AFF-A	AA044	$180
Human β 2 Microglobulin	2mg	AFF	AU043	$180
	1mg	AFF-F	AF043	$140
	1mg	AFF-P	AP043	$160
	0.5mg	AFF-A	AA043	$180
Human Granulocyte Colony Stimulating Factor	2mg	AFF	AU126	$195
	1mg	AFF-F	AF126	$150
	1mg	AFF-P	AP126	$180
	0.5mg	AFF-A	AA126	$260
	1mg	AFF-B	AB126	$180

DESCRIPTION	PACK	GRADE	CODE	PRICE
Human Interleukin 4	0.5mg	AFF	AU143	$120
	0.5mg	AFF-F	AF143	$150
	0.5mg	AFF-P	AP143	$180
	0.5mg	AFF-A	AA143	$220
	0.5mg	AFF-B	AB143	$180
Human Interleukin 6	0.5mg	AFF	AU144	$120
	0.5mg	AFF-F	AF144	$150
	0.5mg	AFF-P	AP144	$180
	0.5mg	AFF-A	AA144	$220
	0.5mg	AFF-B	AB144	$180
Human Interferon γ	0.5mg	AFF	AU138	$120
	0.5mg	AFF-F	AF138	$150
	0.5mg	AFF-P	AP138	$180
	0.5mg	AFF-A	AA138	$220
	0.5mg	AFF-B	AB138	$180
Human Tumour Necrosis Factor (TNF) Alpha	0.5mg	AFF	AU139	$120
	0.5mg	AFF-F	AF139	$150
	0.5mg	AFF-P	AP139	$180
	0.5mg	AFF-A	AA139	$200
	0.5mg	AFF-B	AB139	$180
Human Ferritin (Spleen)	0.5mg	AFF	AU055	$90
	0.5mg	AFF-F	AF055	$95
	0.5mg	AFF-P	AP055	$110
	0.5mg	AFF-A	AA055	$150

[ ] - Affinity Purified Grade

KEY:
- AFF - IgG Fraction
- AFF-F - FITC conjugate
- AFF-P - Peroxidase conjugate
- AFF-A - Alkaline phosphatase conjugate
- AFF-B - Biotin conjugate

the Binding Site 1993

ANTI HUMAN IgG (GAMMA CHAIN) Fab MONOMER - FITC

(For flow cytometry)

APPLICATION:

This reagent is for detecting human immunoglobulin (IgG) on cells by flow cytometry where agglutination must be avoided. It has been particularly designed to detect and quantitate Rh(D) positive foetal cells in Rh(D) negative maternal blood. Using this labelled Fab Monomer as a second antibody after incubation with human anti-D, is becoming established as an alternative and more accurate procedure than conventional acid elution staining techniques.

PREPARATION OF Fab MONOMER:

IgG fraction of sheep anti human IgG is papain digested in the presence of L-Cysteine hydrochloride. The resultant Fab monomer is isolated by gel

The Fab monomer is then conjugated to fluorescein isothiocyanante (FITC). Unreacted fluorescein is removed by gel filtration (Sephadex G25), followed by ion-exchange chromotography to ensure an optimal fluorescein/protein (F/P) ratio.

PRESENTATION:

2.5mg of sheep anti human IgG Fab monomer in 0.5mL PBS, pH 7.2, liquid form containing 0.1% sodium azide.

STORAGE / STABILITY:

2 years from date of manufacture at -20°C.

DESCRIPTION	GRADE	CODE	PRICE
Anti Human IgG Fab			

Anti-Human Tumor Necrosis Factor-alpha, monoclonal

250 micrograms **Catalog #05-106**

Source

mouse-mouse hybridoma (designation 2-2-3E3 (P3-X63-Ag8.653 myeloma x BALB/c spleen cells)), propagated as mouse ascites; immunogen is human recombinant TNF-alpha

Characteristics

Immunoglobin Type:
IgG1

Purification Method:
DEAE-Sepharose chromatography

Specificity:
recognizes human tumor necrosis factor-alpha (cachectin); reacts with tumor necrosis factor-alpha in dog tissue in immunocytochemical application

Formulation:
250 micrograms IgG1 in 0.01 M sodium phosphate, pH 8.0/0.15 M sodium chloride; vialed aseptically; frozen

Stability:
1 year at -20°C
1 month at 4°C

Applications

Western Immunoblotting:
use 10 micrograms/ml to detect 100 nanograms of human recombinant TNF-alpha, with higher sensitivity under non-reducing conditions

Neutralization of TNF-alpha:
use 100 to 200 nanograms/ml to effect 50% neutralization of the biological activity of 20 nanograms TNF-alpha per ml in the mouse L929 fibroblast cytolytic/cytotoxic assay

Immunocytochemistry:
dilute 1:50 on frozen sections of unfixed tissue (see Protocol Section)

Reference

Aggarwal et al., J. Biol. Chem. 260: 2345, 1985

Anti-Mouse Tumor Necrosis Factor-alpha, monoclonal

500 micrograms **Catalog #05-168**

Source

mouse-rat hybridoma (designation MP8-XT22 (P3X63Ag8.653 mouse myeloma x Lewis rat splenocytes)) propagated in serum-free cell culture; immunogen is mouse recombinant Tumor Necrosis Factor-alpha

Characteristics

Immunoglobin Type:
IgG1

Purification Method:
HPLC; displays two bands (heavy and light chains) on SDS-PAGE under reducing conditions

Specificity:
binds and neutralizes mouse TNF-alpha; does not recognize human TNF-alpha

Formulation:
500 micrograms IgG1 in carrier-free PBS; vialed aseptically; lyophilized

Rehydration:
in 500 microliters water; dilute further as required with PBS or other physiological buffer

Stability:
lyophilized: 2 years at 4°C
renydrated: 1 year at -20°C

Applications

Western Immunoblotting:
use 1 microgram/ml to detect 100 nanograms mouse TNF-alpha

Dot Blot:
use 1 microgram/ml to detect 1 nanogram mouse TNF-alpha

Biological Neutralization:
recommended

URI
1994

A[illegible] Mouse GM-CSF Monoclo[illegible]

Ordering code:
MM-500D

Size:
500 ug / vial

Source:
Purified from mouse ascites fluid.

Isotype:
Rat $IgG_{1}\kappa$

Clone Number:
MP1-31G6

Specificity:
Specific for natural and recombinant mouse GM-CSF. will not bind human GM-CSF.

Antigen:
Recombinant mouse GM-CSF.

Activity:
Neutralizing. Certificate of Analysis included with each shipment.

Formulation/Concentration:
Provided as purified antibody in preservative- and carrier-free PBS at 500 ug / vial in a volume of 500 ul.

Purification:
Ammonium Sulfate Precipitation

Endotoxin:
<12 EU/mg.

Stability:
Shipped on cold packs. To ensure stability aliquot and freeze at -20°C or below immediately upon receipt. Stable until expiration date if stored under these conditions.

Applications:
Immunoprecipitation.
Western Blot: Use in the range of 5-10 ug/ml. *Caution.* for the detecting system. do not use a labled Protein A or Protein G. use a labeled anti-rat IgG.
ELISA: As a detecting antibody in a sandwich ELISA use in the range of 5-10 ug/ml in PBS. Use with Endogen product MM-500C.

SELECTED REFERENCES:
1. Abrams, J.A., et al., 1992. *Immunological Rev.* 127(1):5.
2. Vannuccini, A.M., et al., 1990. *Blood.* 76:1473.
3. Kreider, B.L., et al., 1990. *Mol. Cell. Biology.* 10:4846.
4. Ulich, T.R., et al., 1990. *Am. J. Pathology.* 137:369.
5. Keith, W.N., et al., 1990. *Brit. J. Cancer.* 62:388.
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Anti-Human Tumor Necrosis Factor alpha (TNFα) Monoclonal

Ordering code:
M-300A

Size:
500 ug / vial

Source:
Purified from mouse ascites fluid.

Isotype:
Mouse $IgG_{1}\kappa$

Specificity:
Specific for natural and recombinant human TNFα.

Antigen:
Recombinant human TNFα.

Activity:
Neutralizing antibody as determined in the L929 cytotoxicity assay, see Cytokine Properties section for complete procedure. Lot specific Certificate of Analysis included with each shipment.

Formulation/Concentration:
Provided as purified antibody in preservative- and carrier-free PBS at 500 ug/vial in a volume of 500 ul.

Endotoxin:
<12 EU/mg.

Stability:
Shipped on cold packs. To ensure stability aliquot and freeze at -20°C or below immediately upon receipt. Stable until expiration date if stored under these conditions.

Applications:
Western Blot: Use in the range of 5-10 µg/ml.
ELISA: As a detecting antibody in a sandwich ELISA use in the range of 5-10 ug/ml in PBS. Use with Endogen product M-301.

SELECTED REFERENCES:
1. E.A. Carswell, et al., 1975. *Proc. Nat'l Acad. Sciences. USA.* 72: 3666.
2. D. Pennica, et al., 1984. *Nature* 312:724.
3. B. Beutler, et al., 1985. *Science.* 229: 869.
4. B Beutler, et al., 1985. *Nature.* 316:552.
5. Michie, H.R., et al., 1988. *J. Medicine.* 318(23).

Endogen 1994 cat

Anti-Human Tumor Necrosis Factor alpha (TNFα) Monoclonal

Ordering code:
M-301

Size:
500 ug / vial

Source:
Purified from mouse ascites fluid.

Isotype:
Mouse $IgG_{1\kappa}$

Specificity:
Specific for natural and recombinant human TNFα.

Antigen:
Recombinant human TNFα.

Activity:
Neutralizing antibody as determined in the L929 cytotoxicity assay, see Cytokine Properties section for complete procedure. Lot specific Certificate of Analysis included with each shipment.

Formulation/Concentration:
Provided as purified antibody in preservative- and carrier-free PBS at 500 ug/vial in a volume of 500 µl.

Purification:
Protein A

Endotoxin:
<12 EU/mg.

Stability:
Shipped on cold packs. To ensure stability aliquot and freeze at -20°C or below immediately upon receipt. Stable until expiration date if stored under these conditions.

Applications:
Western Blot: Use in the range of 5-10 ug/ml.
ELISA: As a coating antibody in a sandwich ELISA use in the range of 5-10 µg/ml in PBS. Use with Endogen product M-300A.

SELECTED REFERENCES:
1. E.A. Carswell, et al., 1975. *Proc. Nat'l Acad. Sciences. USA.* 72: 3666.
2. D. Pennica, et al., 1984. *Nature.* 312:724.
3. B. Beutler, et al., 1985. *Science.* 229: 869.
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5. Michie, H.R., et al., 1988. *J. Medicine.* 318(23).

Anti-Human Tumor Necrosis Factor alpha (TNFα) Polyclonal

Ordering code:
P-300A

Size:
1 mg / vial

Source:
Purified from the serum of rabbits.

Specificity:
Specific for natural and recombinant human TNFα. Does not cross react with mouse or rat TNFα.

Antigen:
Recombinant human TNFα.

Activity:
Neutralizing antibody as determined in the L929 cytotoxicity assay, see Cytokine Properties section for complete procedure. Lot specific Certificate of Analysis included with each shipment.

Formulation/Concentration:
Each vial contains 1.0 mg highly purified IgG in 1.0 ml PBS buffer. pH 7.4. This reagent is preservative- and carrier-free.

Purification:
Protein A

Endotoxin:
<12 EU/mg.

Stability:
Shipped on cold packs. To ensure stability aliquot and freeze at -20°C or below immediately upon receipt. Stable until expiration date if stored under these conditions.

Applications:
Western Blot: Use at 10 µg/ml with 100 ng of cytokine per lane.
ELISA: Use in the range of 5-15 µg/ml in PBS.

SELECTED REFERENCES:
1. Benjamin, D., et al., 1992. *Lymph. Cytok. Res.* 11(1):45.
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3. Lazanov, A.W., et al., 1992. *Cytokine.* 4(6):479.
4. Maslenczyk, J., et al., 1992. *J. Immunology.* 149(8):2702.
5. Smyth, M.R., 1992. *Immun. Cell. Biol.* 70:379.
6. Dinarello, C.A., et al., 1986. *J. Exp. Med.* 163:1433.
7. Aggarwal, B.B., et al., 1985 *J. Bio. Chem.* 260: 2345.
8. Beutler, B., et al., 1985. *Nature.* 316:552.
9. Pennica, D., et al., 1984. *Nature.* 312:724.
10. Carswell, E.A., et al., 1975. *Proc. Nat'l. Acad. Sci., USA.* 72:3666.